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Effects of Cations on the Volume and Elemental Composition of Nematocysts Isolated from Acontia of the Sea Anemone *Calliactis polypus*

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Abstract. The hypothesis that exchange of intracapsular divalent cations with Na^+ in seawater increases the internal osmotic pressure during discharge of nematocysts of marine cnidarians was tested by examining effects of externally applied cations on the volume and elemental composition of nematocysts isolated from acontia of the sea anemone *Calliactis polypus*. The volume of isolated nematocysts increased with increasing concentrations of cations if the cation was monovalent but appeared to decrease if the cation was divalent. Ca^{2+} reduced the internal osmotic pressure of the nematocysts more efficiently than Mg^{2+}. X-ray microanalysis of nematocysts incubated in 1 *M* solutions of various salts showed that Ca^{2+} in isolated nematocysts was only partially replaced, if at all, by externally applied Na^+ and Mg^{2+} while most Mg^{2+} was replaced by Na^+ and Ca^{2+}. The present results suggest that exchange of intracapsular divalent cations with external monovalent cations increases the internal osmotic pressure, and that selective binding of Ca^{2+} to polyanions in the capsule decreases it. Whether the increase in the internal osmotic pressure caused by the cation exchange is large enough to trigger discharge remains to be investigated.

Introduction

Lubbock and his colleagues proposed that loss of Ca^{2+} from a nematocyst increases the osmotic pressure of the intracapsular fluid and thus causes discharge of the nematocyst (Lubbock and Amos, 1981; Lubbock *et al.*, 1981; Gupta and Hall, 1984). They proposed that polypeptides in undischarged nematocysts are crosslinked by Ca^{2+} to form polypeptide chains and that the release of calcium from the nematocyst dissociates the polypeptide chains, thereby increasing the number of osmotically active molecules. Because of this report, Ca^{2+} has been considered to play a major role in nematocyst discharge.

Recently Weber (1989) demonstrated that naturally occurring cations of *Hydra* nematocysts can be replaced by externally applied cations. Nematocysts loaded with other cations generally retain discharge capabilities. Gerke *et al.* (1991) found that *in situ* nematocysts of *Hydra* contained high concentrations of potassium (K) instead of calcium (Ca). These observations suggest that Ca^{2+} is not indispensable for the discharge of certain kinds of nematocysts.

Weber (1989) proposed that *Hydra* nematocysts can be considered as Donnan-equilibrium dominated osmotic systems and that cations associated with polyanions in the capsule, rather than polyanions themselves, contribute to high intracapsular osmotic pressure. Because *Hydra* nematocysts contain high concentrations of K (Gerke *et al.*, 1991) and are surrounded by a membrane that might serve as a diffusion barrier against ions of low molecular weight (Lubbock *et al.*, 1981), nematocysts of *Hydra* might be in equilibrium with high concentrations of K^+. If such nematocysts are exposed to freshwater as a result of exocytosis, the osmotic pressure difference across the capsule wall would increase, leading to the discharge of the nematocysts. Indeed, isolated *Hydra* nematocysts immersed in concentrated NaCl or KCl solutions swell up to 115% of the original volume and tend to discharge when the external concentration of the salts is lowered (Weber, 1989).

The above process, however, may not account for the discharge of nematocysts of marine cnidarians, because nematocysts of marine cnidarians must discharge in seawater, which contains high concentrations of salts. X-ray

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microanalysis of frozen sections of various marine cnidarians show that the predominant cation of nematocysts *in situ* is either Ca^{2+}, Mg^{2+}, or K^+ (Tardent *et al.*, 1990). If nematocysts of marine cnidarians also behave as Donnan-equilibrium dominated systems, exchange of intracapsular cations with cations in seawater will occur when nematocysts are exposed to seawater as a result of exocytosis. In Ca- or Mg-containing nematocysts, the exchange of divalent cations in the capsule with monovalent cations such as Na^+ in seawater might increase the internal osmotic pressure, since one divalent cation is replaced by two monovalent cations to maintain electroneutrality. If the increase in the internal osmotic pressure is large enough, the nematocysts would discharge.

The purpose of the present study is to examine the hypothesis of divalent-monovalent cation exchange. Undischarged nematocysts isolated from various cnidarians contain high concentrations of Ca and Mg (Weber *et al.*, 1987; Mariscal, 1988; Hidaka, 1993). These isolated nematocysts provide a useful model for studying the responses of Ca- and/or Mg-containing nematocysts to various cations. We determined the effects of mono- and divalent cations on the volume of nematocysts isolated from acontia of the sea anemone *Calliactis polypus*. We also studied whether Ca^{2+} and Mg^{2+} found in the isolated nematocysts could be replaced by externally applied cations as in *Hydra* nematocysts (Weber, 1989).

Materials and Methods

Specimens of *Calliactis polypus* on the shells of hermit crabs belonging to the genus *Dardanus*, were collected from the reef around the Okinawa island, and maintained in an aquarium supplied with a subgravel filter. The hermit crabs were fed with chopped *Tapes* every 2–4 days. The anemones were used as a source of acontial nematocysts 1–3 days after feeding. Acontial filaments were obtained by prodding the sea anemone with blunt-tipped forceps.

Undischarged basitrichous isorhiza nematocysts were isolated either in artificial seawater (ASW) or in distilled water (DW), since nematocysts isolated in ASW and those isolated in DW display different discharge capabilities (Hidaka and Mariscal, 1988). A piece of acontium was placed in a drop of ASW or DW on a glass slide. The glass slide was treated with a drop of a 0.1% solution of poly-l-lysine (Sigma; approx. mol. wt. 90,000) in distilled water for 10 min in a wet chamber prior to use (Mazia *et al.*, 1975). Two strips of thin adhesive tape (Scotch 3M) were placed on both sides of the drop to make a narrow space between the glass slide and a cover slip, and to make it easy to replace the solution in this space. When nematocysts were isolated in ASW, the acontium was squashed under a cover slip. Only a small percentage (about 5%) of nematocysts discharged during this procedure, and most of them only partially discharged—eversion of the tubule stopped halfway. Cellular debris and partially discharged nematocysts were removed by washing the squashed acontium with a few drops of ASW. Most of the nematocysts isolated in this manner from acontia of *Calliactis tricolor* discharged when immersed in 5 m*M* EGTA (Hidaka and Mariscal, 1988), suggesting that the isolated nematocysts are functional. When nematocysts were isolated in DW, the acontium was immersed in a drop of DW for 5 min and then the remaining acontium was removed. The extruded undischarged nematocysts were allowed to settle onto the glass slide for 10 min. Then, the isolated nematocysts were washed with more than five drops of ASW or DW to remove unattached nematocysts.

Photomicrographs of nematocysts were taken in ASW or DW using a plan objective lens (×100). Then, test solutions were applied by perfusing the nematocysts with at least eight drops of each test solution (Hidaka and Mariscal, 1988). Nematocysts isolated in ASW were treated with decreasing concentrations of salt solutions, that is, 1000, 100, 10, 1, and 0 m*M* solutions. Nematocysts isolated in DW were treated with increasing concentrations of salt solutions. Nematocysts were immersed in each test solution for 10 min, because changes in the volume of isolated nematocysts in solutions with or without Ca^{2+} were completed within 10 min (Hidaka, 1992). After 10 min a pair of photomicrographs of the nematocysts was taken for each test solution. The length and diameter of nematocysts capsules were measured to the nearest 0.1 mm (corresponding to 0.05 μm) on two photomicrograph prints using calipers. The average value was used for each capsule. The volume was calculated assuming that the capsule was an ellipsoid. The volume of nematocysts immersed in test solutions was normalized by the original volume of the nematocysts in ASW or DW, and expressed as relative volume. The original volume (mean ± SD) was 117.7 ± 18.8 μm^3 (n = 37) in ASW-isolated nematocysts and 100.7 ± 10.1 μm^3 (n = 37) in DW-isolated nematocysts. For each salt solution, three or four experiments were performed and at least seven nematocysts were measured. The significance of regression of the relative volume of nematocysts on log (salt concentration) was tested in each salt solution. When the regression was not significant, the relative volume at 1 *M* salt concentration was compared with that at 1 m*M* using Duncan's multiple range test. The difference in the relative volume of nematocysts was tested among pairs of cations at 1 *M* salt concentration using the multiple range test.

For substitution experiments, nematocysts were isolated by immersing 20 acontia in 5 ml of DW for 5–10 min. Remaining acontial tissues were removed by filtering the nematocyst suspension through 60 μm nylon mesh. Aliquots (0.8 ml) of the filtrate were placed in each of six

microtubes and centrifuged at 1940 × *g* for 5 min. One ml of each test solution was added to the pellet. Nematocysts were resuspended in the test solutions and allowed to stand for 10 min. Test solutions were ASW and 1 *M* solutions of NaCl, KCl, $CaCl_2$, $MgCl_2$, and $SrCl_2$. Next, the nematocysts were washed in DW by centrifuging at 1940 × *g* for 5 min and by resuspending the nematocysts in 1 ml of DW. The nematocysts were washed in DW and collected by centrifugation three times. Finally, the nematocysts were resuspended in 150 μl of DW. Aliquots (20 μl) of the nematocyst suspension were placed on meshes with formval or collodion membranes that had been coated with carbon and treated with poly-l-lysine. The nematocysts were allowed to settle on the membrane for 1 h, then air-dried after the remaining solution was soaked up with a piece of filter paper. Specimens were observed under a scanning transmission electron microscope (JEOL JEM-2000EX) equipped with an energy dispersive spectrometer (TN 421J). X-ray spectra were acquired at an acceleration voltage of 100 kV. Semiquantitative elemental analyses were performed using an application software (Noran Instruments Inc. SMTF) on 4–6 nematocysts for each test solution. The software, which was designed for standardless semiquantitative analysis of metallurgical thin films, removes background and integrates peak areas. The peak intensities were converted to ratios of element concentrations by multiplying by calculated K-factors. Correction for absorption was not made.

The ASW contained (in m*M*): NaCl, 480; KCl, 10; $CaCl_2$, 10; $MgCl_2$, 26; $MgSO_4$, 29; and was adjusted to pH 8.0 with 10 m*M* HEPES. All the salt solutions and DW were buffered to pH 8.0 with 10 m*M* HEPES, and all the experiments were done at room temperature (23–26°C).

Results

Nematocysts isolated from acontia of *Calliactis polypus* in ASW swelled in concentrated solutions of monovalent cations (Fig. 1). There was a significant positive regression between the volume of nematocysts and the concentration of Na^+ and K^+ (regression analysis, $P < 0.05$). Though there was no significant regression between the volume of nematocysts and the concentration of divalent cations, the volume of nematocysts was significantly smaller in 1 *M* $MgCl_2$ and $SrCl_2$ than in 1 m*M* solutions (Duncan's multiple range test, $P < 0.01$). At 1 *M* concentration, nematocysts immersed in divalent cations were significantly smaller than those immersed in monovalent cations (Duncan's multiple range test, $P < 0.01$). When nematocysts that had been immersed in various salt solutions were immersed in a buffer solution without added salts, there was no significant difference in the mean volume

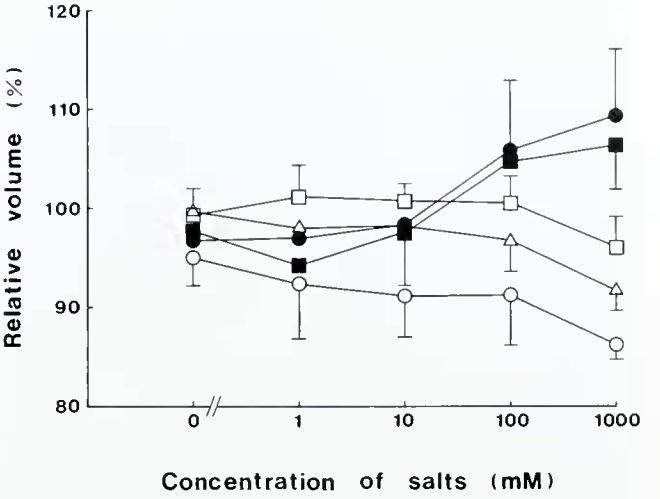

Figure 1. Effects of cations of various concentrations on the volume of nematocysts isolated from acontia of *Calliactis polypus* in ASW. Nematocysts isolated in ASW were immersed successively in salt solutions of decreasing concentrations. The salt solutions examined were NaCl (●), KCl (■), $CaCl_2$ (○), $SrCl_2$ (△), and $MgCl_2$ (□). The volume of nematocysts in each solution is expressed as a percentage of the original volume of nematocysts in ASW. Vertical bars represent standard deviations; some SD bars are omitted for clarity.

(one-way ANOVA, $P > 0.25$). Thus the volume of the nematocysts increased with increasing concentration of cations if the cation was monovalent, but decreased if the cation was Mg^{2+} or Sr^{2+}. The volume of the nematocysts was smaller in 1 *M* $CaCl_2$ than in 1 *M* $MgCl_2$ ($P < 0.01$).

The volumetric behavior of nematocysts isolated in DW was almost the same as that of nematocysts isolated in ASW (Fig. 2). When the concentration of external K^+ was increased, the volume of isolated nematocysts increased (regression analysis, $P < 0.05$). Though regression between the volume of nematocysts and concentration of Na^+ was not significant, nematocysts immersed in 1 *M* NaCl were larger than those immersed in 1 m*M* NaCl (Duncan's multiple range test, $P < 0.05$). Nematocysts immersed in 1 *M* $CaCl_2$ and $SrCl_2$ were smaller than those immersed in 1 m*M* solutions ($P < 0.01$). Nematocysts immersed in 1 *M* $CaCl_2$ or $SrCl_2$ were smaller than those immersed in 1 *M* $MgCl_2$ ($P < 0.01$).

A scanning electron micrograph of a nematocyst sample prepared for X-ray microanalysis is shown in Figure 3. X-ray spectra of nematocysts were different depending on the incubation solutions (Fig. 4). The major elements of nematocysts incubated in ASW were Ca and Mg in addition to sulfur (S), though a small Na-peak was present (Fig. 4A). When nematocysts were incubated in 1 *M* NaCl, the Na-peak increased, the Ca-peak remained high but the Mg-peak disappeared (Fig. 4B). When nematocysts were immersed in 1 *M* KCl, a small K-peak appeared, but the peaks of the other elements were not affected (Fig.

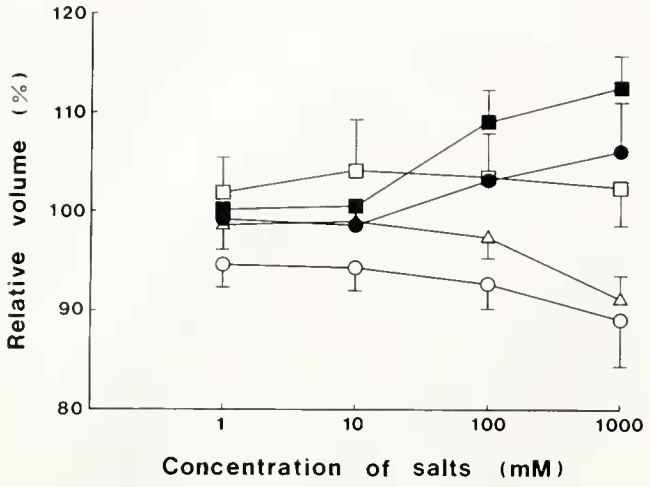

Figure 2. Effects of cations of various concentrations on the volume of nematocysts isolated from acontia of *Calliactis polypus* in DW. Nematocysts isolated in DW were immersed successively in salt solutions of increasing concentrations. The symbols are the same as in Figure 1. The volume of nematocysts in each solution is expressed as a percentage of the original volume of the nematocysts in DW. The vertical bars represent standard deviations; some SD bars are omitted for clarity.

4C). Nematocysts incubated in 1 *M* $CaCl_2$ showed large Ca- and small Na-peaks in addition to the S-peak (Fig. 4D). When nematocysts were incubated in 1 *M* $MgCl_2$, the Mg-peak increased (Fig. 4E). Nematocysts incubated in 1 *M* $SrCl_2$ showed large Sr- and small Ca-peaks in addition to the S-peak (Fig. 4F). When discharged nematocysts were analyzed, peaks of metals were absent regardless of the incubation solutions.

Table I shows the relative abundance of metal cations in undischarged nematocysts that were isolated in DW and then incubated in various salt solutions. Ca accounted for about 50% of the metals in nematocysts immersed in ASW or 1 *M* $MgCl_2$ and more than 50% in nematocysts immersed in 1 *M* NaCl or KCl. Ca was replaced substantially only by strontium (Sr). Most of the Mg disappeared when nematocysts were immersed in 1 *M* NaCl, $CaCl_2$, and $SrCl_2$. Only a small amount of K was present in nematocysts incubated in 1 *M* KCl.

Discussion

Weber (1989) studied the volumetric behavior of isolated stenoteles of *Hydra* under different ionic conditions. He showed that nematocysts immersed in 1 *M* solutions of various salts swell when the concentration of salts is lowered, regardless of whether the cations are monovalent or divalent. The volumetric behavior of isolated nematocysts of the sea anemone *Calliactis polypus* was different from that of *Hydra* nematocysts. *Calliactis* nematocysts appeared to shrink in concentrated solutions of divalent cations as in *Hydra* nematocysts, but swelled in concentrated solutions of monovalent cations. Thus the volumetric behaviors of the marine anemone nematocysts and the freshwater *Hydra* nematocysts are different in solutions of monovalent cations.

Weber (1989) showed that the volumetric behavior of *Hydra* nematocysts immersed in salt solutions of various concentrations can be accounted for by a Donnan-equilibrium model. The Donnan potential generates an asymmetrical distribution of ions across the capsule wall. According to Weber's simulation studies, the difference in total ion concentration between the inside and outside of the capsule increases as the external salt concentration is lowered from 3 *M* to 0.1–0.01 *M*. When the external salt concentration is further lowered, the osmolarity difference drops due to protonation of polyanions, unless the external pH is high. The volume of *Calliactis* nematocysts, however, decreased as the external concentration of monovalent cations was lowered from 1 to 0 *M*. Thus the volumetric response of the sea anemone nematocysts to monovalent cations cannot be accounted for by the simple Donnan-equilibrium model.

Weber (1989) showed that naturally occurring cations in *Hydra* nematocysts can be replaced by externally applied cations. If this is true for nematocysts of marine cnidarians, cations contained in the isolated capsule might be replaced by external cations when nematocysts are immersed in various salt solutions. Isolated *Calliactis* nematocysts contained predominantly Ca^{2+} and Mg^{2+} (Hidaka, 1993). If Ca^{2+} and Mg^{2+} in the isolated nematocysts are replaced by monovalent cations, the internal osmotic pressure would increase as one divalent cation is replaced by the two monovalent cations required to maintain electroneutrality. The swelling of the sea anemone nemato-

Figure 3. Scanning electron micrograph of isolated nematocysts used for X-ray microanalysis. The white spot represents the site irradiated with an electron beam during the acquisition of spectra. These nematocysts were isolated in DW and incubated in 1 *M* $MgCl_2$ for 10 min.

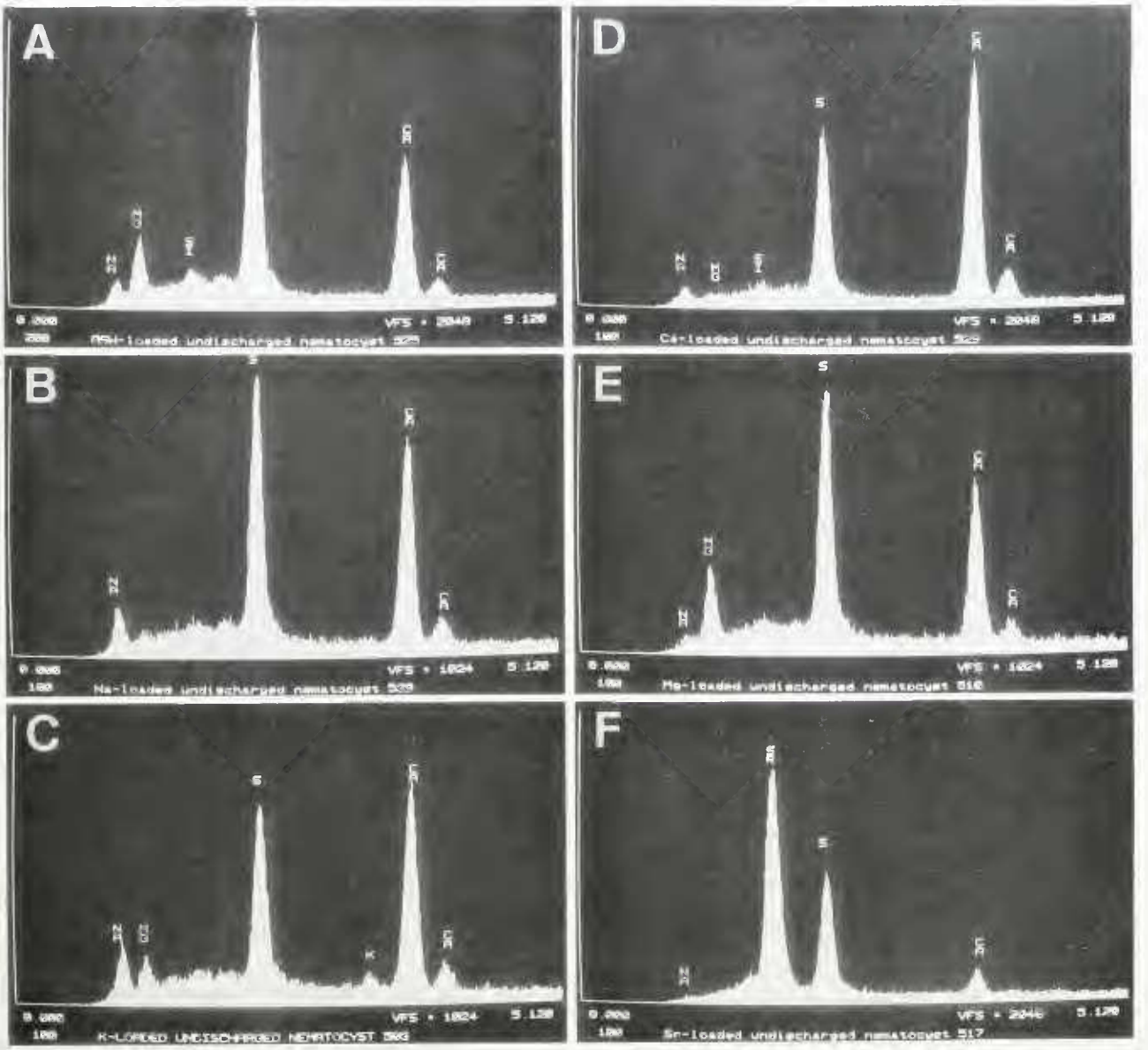

Figure 4. X-ray spectra of nematocysts incubated in various salt solutions. Nematocysts isolated in DW were incubated in ASW or 1 *M* solutions of various salts for 10 min. A, ASW. B, NaCl. C, KCl. D, $CaCl_2$. E, $MgCl_2$. F, $SrCl_2$.

cysts in 1 *M* NaCl or KCl might be partly due to an exchange of intracapsular divalent cations with monovalent cations in the bathing solution.

X-ray microanalysis of *Calliactis* nematocysts incubated in 1 *M* solutions of various salts for 10 min showed that the predominant cation in the nematocysts was not necessarily the cation in the incubation medium. The predominant cation in nematocysts incubated in 1 *M* NaCl, KCl, $MgCl_2$ and $CaCl_2$ was Ca^{2+}. Beside Ca^{2+}, Sr^{2+} is the only cation that replaced most of the cations in isolated nematocysts and accounted for about 90% of the cations contained in the nematocysts. This implies that Ca^{2+} in isolated nematocysts was replaced only partially, if at all, by externally applied cations. This contrasts with the observation that Ca^{2+} and Mg^{2+} found in isolated *Hydra* nematocysts can be replaced almost completely by externally applied cations (Weber, 1989). On the other hand, most of the Mg^{2+} in isolated nematocysts is replaced by externally applied Na^{2+}, since Mg^{2+} almost disappeared after incubation in 1 *M* NaCl.

However, it was difficult in this experiment to estimate what percentage of Ca^{2+} and Mg^{2+} in isolated nematocysts was actually replaced by externally applied cations. The present semiquantitative analyses do not provide a mea-

Table I

Relative abundance of metal elements in isolated Calliactis polypus *nematocysts incubated in various salt solutions*[1]

Solutions	Na	K	Ca	Mg	Sr	N[2]
ASW	10.9 ± 5.8	1.4 ± 0.4	52.5 ± 4.7	35.2 ± 2.4	0	5
1 *M* NaCl	26.6 ± 8.3	1.5 ± 0.2	71.7 ± 8.4	0.3 ± 0.4	0	4
1 *M* KCl	23.2 ± 7.1	2.6 ± 1.8	61.3 ± 7.1	13.0 ± 1.9	0	4
1 *M* $CaCl_2$	6.5 ± 6.1	1.6 ± 0.3	91.7 ± 6.3	0.2 ± 0.3	0	6
1 *M* $MgCl_2$	5.9 ± 5.9	0.8 ± 0.4	48.7 ± 5.7	44.5 ± 3.2	0	5
1 *M* $SrCl_2$	0	0	8.4 ± 0.3	0.7 ± 0.5	90.1 ± 0.3	4

[1] The relative abundance of each metal element is shown as a percentage of total metal elements listed. Means ± SD.
[2] Number of nematocysts analyzed.

sure of the absolute concentration of each metal but only their relative abundances. Some of the cations that have lower affinity for polyanions in the capsule might be lost during washing in DW. If this is the case, the actual concentration of Na^+ and K^+ in the nematocysts immersed in 1 *M* solutions of these monovalent cations must have been much higher than estimated. It is likely that most of the Mg^{2+} and some of the Ca^{2+} in isolated nematocysts are replaced by Na^+ or K^+ when the nematocysts are immersed in 1 *M* NaCl or KCl. Such divalent cation-monovalent cation exchange might at least partly account for the swelling of nematocysts in solutions containing high concentrations of monovalent cations.

Nematocysts isolated in ASW and those isolated in DW responded similarly to changes in external salt concentration. Nematocysts isolated in DW swelled as the external concentration of monovalent cations was increased. This is probably because a greater percentage of the divalent cations was replaced by monovalent cations as the concentration of external monovalent cations was raised. The volume of nematocysts isolated in ASW decreased as the concentration of external monovalent cations was lowered. It is unlikely that the decrease in the nematocyst volume was due to an exchange of intracapsular monovalent cations with external divalent cations, since the external solutions did not contain divalent cations. The affinity of divalent cations for polyanions might have become higher, and negative charges on the polyanions might have become neutralized, by tightly bound divalent cations as the ionic strength decreased. This might account for the decrease in the volume of the nematocysts at low monovalent cation concentrations.

Since Ca^{2+} reduced the volume of isolated nematocysts to a greater extent than Mg^{2+}, Ca^{2+} might have a higher affinity for polyanions than Mg^{2+}. The substitution experiments also show that Ca^{2+} had a higher affinity for polyanions than Mg^{2+}. Binding of Ca^{2+} to polyanions may mask some of the negative charges on the polyanions and thus reduce the number of osmotically active cations within the capsule. It is also possible that Ca^{2+} cross-links polyanions to reduce the number of osmotically active particles, as suggested by Lubbock *et al.* (1981). Thus, the osmotic pressure of the intracapsular fluid is determined not only by the Donnan-equilibrium, but also by the selective binding of Ca^{2+} to polyanions in the capsule. The present observations suggest that not only divalent-monovalent cation exchange but also exchange of intracapsular Ca^{2+} with Mg^{2+} in seawater will increase the internal osmotic pressure when Ca-containing nematocysts come into contact with seawater.

Lubbock *et al.* (1981) found high concentrations of Ca in undischarged holotrichous isorhiza nematocysts by X-ray microanalysis of frozen sections of mesenterial filaments of the sea anemone *Rhodactis rhodostoma* and acrorhagi of *Anthopleura elegantissima*. They observed that an influx of Na accompanies the efflux of Ca during discharge. Their observation is consistent with the above hypothesis that the osmotic pressure of the sea anemone nematocysts increases due to the exchange of intracapsular divalent cations with Na^+ in seawater. Lubbock *et al.* (1981) suggested that nematocysts become exposed to seawater at the time of discharge—after exocytotic fusion of the nematocyst membrane with the cell membrane. Robson (1973) observed that nematocysts of *Rhodactis rhodostoma* swell up to 150% of their original size when they are exposed to seawater immediately before discharge. This observation suggests that the osmotic pressure of sea anemone nematocysts increases when nematocysts are exposed to seawater.

Weber (1990, 1991) found that polyanions contained in nematocysts of *Hydra* and various marine cnidarians are poly(γ-glutamic acid)s with various degrees of polymerization. As for the cations associated with the polyanions, there are differences between species and between types of nematocysts. Tardent *et al.* (1990) reported that the predominant cation of marine cnidarian nematocysts is either Ca^{2+}, Mg^{2+} or K^+. The predominant cation of the tentacular and acontial nematocysts of *Calliactis par-*

asitica is K^+ as in *Hydra* nematocysts. The tentacular nematocysts of *Anthopleura elegantissima* and *Actinia equina* contain Mg^{2+} while the acrorhagial nematocysts of these species contain predominantly Ca^{2+} (Tardent *et al.*, 1990). The differences in the dominant cation among nematocysts of marine cnidarians suggest that the hypothesis of divalent-monovalent cation exchange is not applicable to all nematocysts of marine cnidarians but only to those that contain predominantly Ca^{2+} and/or Mg^{2+}.

Potassium was almost absent even in nematocysts incubated in 1 *M* KCl, indicating that K^+ had the lowest affinity for polyanions. This means that K^+ is the ideal cation to generate a high internal osmotic pressure if ionic distribution across the capsule wall is determined by a Donnan-equilibrium. K-containing nematocysts may encounter an osmotic pressure difference large enough to trigger discharge when they come into contact with seawater. If they fail to discharge, the internal osmotic pressure would decrease due to the exchange of intracapsular K^+ with Ca^{2+} and Mg^{2+} in seawater.

The problem with the hypothesis of divalent-monovalent cation exchange, is that exposure of nematocysts to seawater alone does not elicit discharge of the nematocysts, as shown by the fact that undischarged nematocysts can be isolated from marine cnidarians in ASW (*e.g.*, Hidaka and Mariscal, 1988). Yanagita (1959), however, found that nematocysts isolated from acontia of the sea anemone *Haliplanella luciae* in 1 *M* glycerol, discharged when immersed in concentrated salt solutions such as seawater and isotonic NaCl. He also reported that when nematocysts isolated in 1 *M* glycerol are immersed in diluted salt solutions such as 0.03 *M* $CaCl_2$, the nematocysts become unresponsive to concentrated salt solutions that would otherwise elicit discharge of the nematocysts. This suggests that the isolated nematocysts can remain undischarged if the salt concentration of the surrounding medium is increased gradually. During artificial isolation of nematocysts in ASW, changes in the ionic composition of the surrounding medium might be too slow to cause nematocyst discharge. Yanagita (1959) also noted that nematocysts liberated into a salt solution through cytolytic disintegration of acontia often remain undischarged.

Furthermore, nematocysts isolated in ASW did not discharge in 1 *M* NaCl or KCl. This suggests that the increase in the internal osmotic pressure caused by cation exchange may not be large enough to trigger discharge. However, the possibility remains that the rate of change in the ionic composition of the surrounding medium was too slow to trigger discharge, because solution exchange was performed by perfusing the test solution drop by drop. If the "stopper" (a sealing structure of nematocysts) is made of viscoelastic material, whether it fractures or not depends on the rate of deformation. Nematocysts would discharge only when the rate of increase in the internal osmotic pressure exceeds a certain limit. When nematocysts incubated in 1 *M* $CaCl_2$ were treated with 1 *M* NaCl using the present perfusion method, the volume of the nematocysts increased by 18% and 3–5% of them discharged (Hidaka and Afuso, unpub. ob.). It is likely that more nematocysts would discharge if the surrounding medium is changed more rapidly. The observation that isolated nematocysts did not discharge in 1 *M* NaCl or KCl does not necessarily contradict the hypothesis of divalent-monovalent cation exchange.

Nematocysts can be induced to discharge in media that contain no or only small amounts of monovalent cations by reagents that rupture disulfide bonds or chelate calcium (Hidaka, 1993). These reagents seem to induce discharge of nematocysts by weakening the nematocyst "stopper." The question of whether such weakening of the "stopper" is involved in the *in situ* mechanism of nematocyst discharge remains to be investigated.

The present results show that the volumetric behavior of isolated *Calliactis* nematocysts immersed in various salt solutions can be explained by the exchange of intracapsular divalent cations with cations in the external medium and by the selective binding of Ca^{2+} to polyanions in the capsule. It remains unknown whether the increase in the internal osmotic pressure caused by the cation exchange is large enough to trigger discharge, or whether nematocyst discharge involves biochemical modification of structural components such as the nematocyst "stopper."

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